Elucidation of the Function of ABCB5: Dimerization of ABCB5β with ABCB6 and ABCB9 and generation of ABCB5 knockout mice

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The ABCB5 gene encodes several isoforms, including two transporters (i.e., ABCB5FL and ABCB5β) and soluble proteins, such as ABCB5α which has been hypothesized to have a regulatory function. ABCB5FL is a full ABC transporter and is expressed in the testis and prostate, whereas ABCB5β is an atypical half-transporter with a ubiquitous expression pattern. ABCB5β has been shown to mark cancer stem cells in several cancer types. In addition, ABCB5β and ABCB5FL have been shown to play a role in tumorigenesis and multidrug resistance. However, ABCB5β shares its entire protein sequence with ABCB5FL, making them difficult to distinguish. It cannot be excluded that some biological effects described for one transporter may be mediated by the other isoform. Therefore, it is difficult to interpret the available data, and some controversies remain regarding their function in cancer cells.

The implications of having two ABCB5 transporters for various biological processes encouraged us to unravel the ABCB5β dimerization status and the exact role of these transporters in the cell. Using three complementary techniques, nanoluciferase-based bioluminescence resonance energy transfer, co-immunoprecipitation, and proximity ligation assay, we identified two novel heterodimers in melanoma: ABCB5β/B6 and ABCB5β/B9. Both heterodimers could be expressed in High-Five insect cells and ATPase assays revealed that both functional nucleotide-binding domains of homodimers and heterodimers are required for their basal ATPase activity.

To further investigate the pathophysiological role of ABCB5 in melanoma, ABCB5 KO UACC-257 was generated using CRISPR/Cas9. RNAseq analysis of UACC-257 ABCB5 KO revealed that numerous genes involved in oxidative phosphorylation (OXPHOS) were differentially expressed when compared to wild-type cells. The Seahorse XF glycolysis assay revealed an increase in mitochondrial ATP production rate in ABCB5 KO clones. This increase in OXPHOS allowed ABCB5 KO clones to proliferate faster in low-glucose and galactose-supplemented medium, while wild-type UACC-257 cells showed a higher proliferation rate in glucose-supplemented medium.

The physiological role of ABCB5 was investigated in Abcb5-deficient C57BL/6J mice by deleting Abcb5 exon 2, thereby knocking out both forms of ABCB5. The mice were fertile and showed altered bioenergetics and lipid metabolism, as well as changes in their blood composition. Although rescue experiments are currently being performed to decipher which isoform is responsible for these observed phenotypes, this study opens new avenues of investigation into the role of ABCB5 in intermediary metabolism and cancer aggressiveness.